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THE DEVELOPMENT OF *GEASTER VELUTINUS*

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THE DEVELOPMENT OF GEASTER VELUTINUS.

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(With 9 Text-figs.)

So far as I am aware no study of the development of any species of the genus *Geaster*, nor of the related genera *Astraeus* and *Myriostoma* has been undertaken previously.

With a view to working out its development the writer searched for and obtained some twelve developmental stages of the epigaean species *Geaster velutinus* Morg., one of the commonest species of the genus present in New Zealand. These young plants were growing in the vicinity of numerous mature plants in a locality in the forest where the species has, to the writer's knowledge, been present for the past six years. It produces a superficial mycelial subiculum—quite noticeable in some cases, scarcely discernible in others—and upon this the young plants were found.

Specimens were fixed in picro-formol, embedded and sections 5 μ and 10 μ were cut with a rotary microtome. These were stained with iron-alum haematoxylin and light green, and mounted in canada balsam.

Structure of the Mature Plant.

A typical specimen immediately before expansion is globose or ovate, superficial, 2–3 cm. diam., and attached at the base to the mycelial subiculum by a strongly developed mycelial cord. If sectioned the plant is seen to consist of several exterior layers enclosing a central sac. The outer layers subsequently split from the apex downwards into several stellate rays which remain expanded and appressed to the substratum or else become revolute, lifting the plant clear from it. The expanded

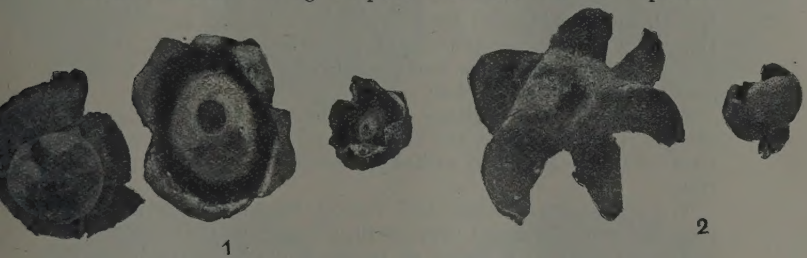

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Fig. 1. *Geaster velutinus* Morg. × ⅔. Saccate specimen in the centre, revolute specimen on the left, expanding plant on the right.

Fig. 2. Exterior of the exoperidium showing the tomentose exterior (more noticeable on developing plant on the right) and prominent umbilical scar—the point of attachment. × ⅔.

plant now has a stellate but somewhat saccate appearance (Fig. 1), and bears in the centre a spore sac which is thin, membranous and perforated apically by a single opening, outlined by a more or less silky or fibrillose zone.

Thus the expanded plant is seen to consist of two well-defined groups of tissues, the stellate part being the exoperidium, the spore sac the endoperidium.

Exoperidium.

The exoperidium consists of three well-defined tissues:

(1) the mycelial or outer layer;

(2) the fibrillose or middle layer; and

(3) the fleshy or inner layer.

(1) *Mycelial layer.* This, the exterior layer, is well seen in unexpanded plants, being in the nature of a dense palisade, 1–2 mm. or more thick, of stout, deeply coloured, persistent hyphae. This layer persists throughout the lifetime of the plant,

and is, in fact, the principal character by which the species is distinguished from several related epigaean species.

(2) *Fibrillose layer.* This consists of intricately woven hyphae of two kinds, arranged with their long axes predominantly radial. The inner portion, next to the fleshy layer, is strengthened by numerous thick-walled hyphae similar to those of the capillitium. The whole tissue is tough and cartilaginous.

(3) *Fleshy layer.* This is pseudoparenchymatous. In freshly expanded plants it is soft, thick and flesh-coloured; after exposure it shrinks considerably, changes to some shade of brown, and often becomes rimose.

Endoperidium.

This structure encloses the gleba, consisting of capillitium and spores, and is attached by a broad base to and partially enclosed by the saccate portion of the exoperidium (Fig. 1). In texture it is membranous, and consists of numerous interwoven, partially gelatinised hyphae.

At the apex is a solitary stoma through which the spores escape. In this species the stoma is surrounded by a zone of parallel fibrils arranged radially, forming a broad, depressed zone.

The spores are globose, dark fuscous brown, strongly verrucose and reticulate, and very small, being 4–4·5 μ diameter.

The capillitium consists of innumerable fusiform, deeply-coloured, simple, continuous hyphae.

Outline of Development.

Development proceeds in the following order:

(1) Differentiation of the mycelial layer of the exoperidium.

(2) Simultaneous differentiation of the fleshy and fibrillose layers, gleba and columella.

(3) Lacunae appear in the gleba, and become lined with a palisade of primary basidia.

(4) Extension of development of lacunae with accompanying palisade of basidia, together with the formation of tramal plates, and later with the appearance of secondary basidia. This is the stage of intense spore production.

(5) The endoperidium becomes differentiated.

(6) Glebal formation (excluding the formation of the capillitium) is concluded.

(7) Peristome and stoma are formed.

(8) Tramal plates of the gleba become broken down and fragmented, and the capillitium grows out from the columella and endoperidium.

(9) The exoperidium becomes revolute and exposes the endoperidium.

Plants are first noticeable when about 2 mm. diam. At this stage they are composed of hyphae of two kinds, macro-hyphae 5–7 μ diam., and micro-hyphae 2–3 μ diam. Both are septate, much branched and intricately interwoven. Tissue differentiation begins shortly after plants attain to this size.

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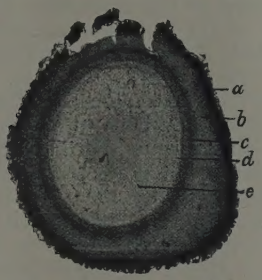

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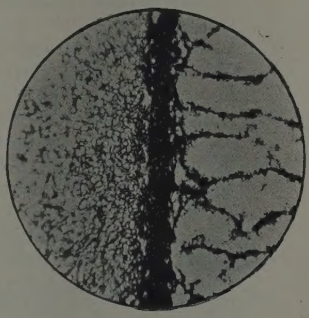

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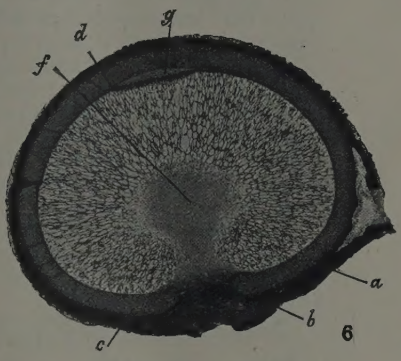

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Fig. 3. Plant showing commencement of differentiation of the gleba and mycelial layer. × 15.

Fig. 4. Later stage showing (*a*) mycelial layer, (*b*) fibrillose layer, (*c*) fleshy layer, (*d*) primordium of the endoperidium, (*e*) glebal mass with lacunae. Section not median. × 10.

Fig. 5. Plant at a later stage; lettering as in the preceding figure, (*f*) columella. Section not quite median. Perforation in the upper portion of the gleba due to insect larva. × 7.

Fig. 6. Median section through plant shortly before collapse of the tramal plates; lettering as above, (*g*) disc of forming peristome. Note that fleshy layer merges abruptly into columella. Wrinkles in fleshy layer formed while section was being affixed to the slide. × 3.

Fig. 7. Photomicrograph of portion of the fleshy layer with the contiguous endoperidium. × 200.

Differentiation of the mycelial layer. In this species a true mycelial layer (as applied to hypogaean species) is wanting, modified hyphae taking its place, forming a tissue which is the first to be differentiated. When plants are approximately 3 mm. diam., numerous dark-walled, unbranched, simple hyphae arise at the periphery directly from the macro-hyphae. They grow radiately from the periphery of the embryonic plant, giving to it a tomentose appearance. As growth proceeds they become elongated, much convoluted, thick-walled, and form a dense layer 1–2 mm. thick, over the exterior (Fig. 4, *a*).

Differentiation of the fibrillose layer. This is first noticeable as a zone of unaltered tissue lying between the mycelial and fleshy layers (Fig. 4, *b*), differentiation beginning about the time the fleshy layer becomes definitely pseudoparenchymatous. At first it consists of both macro- and micro-hyphae, but soon the latter predominate, and become arranged with their long axes radial. Then a third type of hypha makes its appearance, being of the same nature as those forming the mycelial layer, here assuming a position parallel with the latter and serving to strengthen the whole tissue.

The fibrillose layer is of the same nature as primordial tissue (save that micro-hyphae predominate), the fleshy and mycelial layers arising from it as groups of specialised tissues.

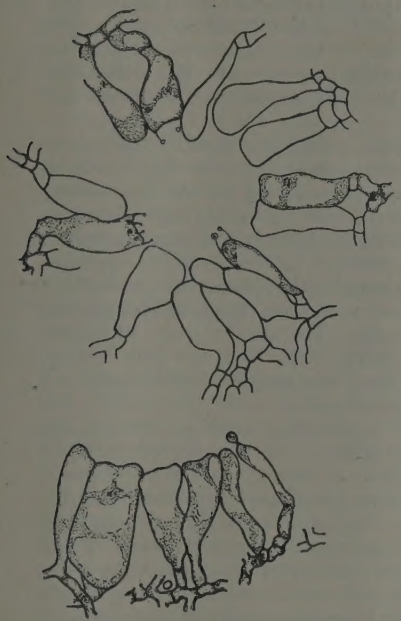
Differentiation of the fleshy layer. Shortly after differentiation of the mycelial layer, the fleshy layer originates as a zone of more closely interwoven and compacted hyphae lying between it and the central region of the plant (primordium of the gleba). This tissue consists almost entirely of macro-hyphae. Soon the hyphal walls become partly gelatinised and the whole becomes pseudoparenchymatous, and is then sharply delimited from the tissues which it encloses and those lying to the exterior (Fig. 4, *c*). It does not form a continuous zone, being interrupted by the thickened base of the columella, into which it abruptly merges (Fig. 6, *c*).

Endoperidium.

This comprises all tissues lying within the fleshy layer, and consists of the spore sac with its contents. In systematic papers it is usual to confine the term endoperidium to the spore sac alone, the capillitium and spores collectively being termed the gleba. This practice will be followed herein.

Gleba. At first this consists of undifferentiated primordium (Fig. 3), but shortly after differentiation of the fleshy layer begins, a dome of lacunae appears between the fleshy layer and the centre of the glebal mass (Fig. 4, *e*), and partially surrounding a non-lacunate region—the columella. The lacunae

arise as simple cavities in the glebal tissue. They vary in size and shape and appear to be formed by the tearing apart of the hyphae. They soon become lined with inflated cells of a size much greater than those of the surrounding hyphae. These inflated cells are in reality basidia, but as they differ greatly from those produced at a later stage they will be termed primary



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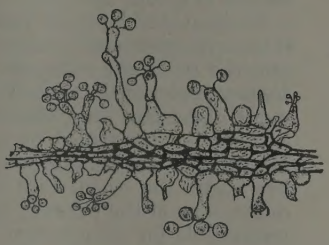

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Fig. 8. Primary basidia. Camera lucida drawing showing the arrangement of basidia around a lacuna in a section 5μ thick. Monosporous basidium in lower figure. × 500.

Fig. 9. Secondary basidia. Camera lucida drawing of portion of a tramal plate showing the gelatinised tissue of the plate (in black), basidia, sterigmata and spores. × 800.

basidia (Fig. 8). Owing to enlargement they compress the intervening tissues (between lacunae) thus giving rise to plates of tissue termed tramal plates (Fig. 6).

The primary basidia are irregular in size and shape and form a close palisade or hymenium lining the lacunae. They arise directly from modified portions of the hyphae of the tramal plates, a sub-hymenium, characteristic of most members of the

Agaricales, being absent. Many become monosporous shortly after their formation (Fig. 8), a condition not uncommon in certain other Gasteromycetes at an early stage in their development. Soon, however, the primary basidia become more regular in shape and bear several spores (usually four at this stage) on short sterigmata.

Next follows a period of rapid formation of lacunae and production of basidia, until the whole of the tissue enclosed within the fleshy layer (or rather the endoperidium, which appears shortly before this active period begins), save the columella, becomes converted into partly gelatinised tramal plates and basidia (Fig. 6).

Basidial development continues rapidly. Each basidium as it matures produces four to eight spores; these become detached, and the basidium collapses, to be replaced by another arising from a hypha of the tramal plate. Owing to this rapid development of basidia the plates become thinner and more lamellar, a change accompanied by a decrease in the size of the basidia. Decrease in size is rapid, so that shortly after spore production has begun the basidia are seen to be less than one-fourth the dimensions of the primary basidia. The smaller ones, which are responsible for the greatest spore production, are here termed secondary basidia (Fig. 9). They are usually four-spored, but eight-spored forms are not uncommon.

This period of intense spore production continues until finally little remains of the tramal plates but strands of partially gelatinised hyphae, with a few adhering, partially collapsed secondary basidia. Then the plates become fragmented and disappear, and at the same time the capillitium threads—hyphae of the same type as those comprising the mycelial layer—make their appearance. These arise principally from the columella; and in lesser numbers from the inner surface of the endoperidium. In consequence the columella becomes smaller as development of the capillitium threads continues, until in the mature plant often scarcely more than a rudiment remains.

The capillitium threads are long, unbranched (save occasionally at the apices), dark in colour, thick walled and acuminately pointed. Thus at maturity the tramal plates have disappeared, the whole of the interior being occupied by the capillitium and spores.

Spore production begins when the plants are 7–8 mm. in diameter, and continues uninterruptedly until the tramal plates break up.

The endoperidium consists of micro-hyphae identical with those of the fibrillose layer. The hyphae become closely and intricately interwoven and partly gelatinised; at first they are

attached both to the tramal plates on the interior and to the fleshy layer on the exterior, but shortly after the endoperidium is clearly defined, partial separation is affected by the tearing apart of the hyphae from the fleshy layer (Fig. 7). When the exoperidium becomes revolute, separation occurs along the junction of the fleshy layer and endoperidium, particles of hyphae persisting for some time as a pruinose covering on both the interior of the one and the exterior of the other.

Peristome. Shortly after the endoperidium has been formed, formation of the peristome begins. The hyphae at the apex of the endoperidium become more loosely interwoven and form a thickened apical disc (Fig. 6, *g*). The hyphae of the disc then assume a radial arrangement, a small irregular cavity appears in the centre and becomes lined with hyphae from the inner portion of the endoperidium. Then the disc collapses, becomes depressed and gives rise to the peristome, which now appears as a circular depressed region perforated by a circular orifice—the stoma (Fig. 1).

Finally rupture of the exoperidium occurs, and the endoperidium is exposed.

Cytology.

The cytology of this species is apparently simple. The hyphae are uninucleate and the nuclei exceedingly small, ranging in size from 0·5 to 1·0 μ in diameter. They are usually found in the vicinity of the septa. The basidia—both primary and secondary—also are uninucleate, the nuclei being about the same size as those of the hyphae. Owing to the exceedingly small size of the nuclei, no chromosomes have been observed.

The behaviour of the nucleus within the basidium during spore production is apparently similar to that of certain other uninucleate Basidiomycetes, the plant appearing to be haploid throughout. The fusion nucleus present in the basidia of *Secotium* and certain species of the Agaricales prior to spore production has not been observed, for at all stages of its development prior to spore production the basidium is uninucleate.

Shortly after the basidium is formed sterigmata grow from the apex. When these are about 3 μ long terminal inflations appear—the developing spores. Until this period the nucleus remains unchanged, but with the beginning of spore formation it rapidly changes, and divides until the number of nuclei are equal to the number of spores produced. Such division may occur in any part of the basidium, but as the sterigmata are developing the nucleus usually takes up a position near the apex of the basidium and there divides. Four is the usual number of spores produced, consequently it is usual to find

this number of nuclei formed by successive divisions of the nucleus. Actual migration has not been observed, owing, no doubt, to the exceeding fineness of the sterigmata (0·25 μ or less) but spores of a diameter of less than 2 μ are seen to possess no nuclei whereas others larger than this are invariably and constantly uninucleate.

At an early stage of development the epispore is quite smooth, but shortly after migration of the nucleus it becomes minutely roughened, and as the spores enlarge the degree of roughness increases until at maturity they are seen to be closely covered with fine, flat-topped verrucae. Immediately after its detachment from the basidium the spore is seen to be shortly pedicellate—due to the persistence of a portion of the sterigma, but this structure apparently disappears, for in spores from a mature plant it is not noticeable.

As all attempts to germinate spores have failed, it has not been possible to observe nuclear behaviour during germination, nor to ascertain whether the first hyphae produced are uninucleate. This peculiarity of non-germination under all laboratory conditions is apparently common in the Lycoperdaceae, for the writer has failed in all attempts to germinate spores of *Bovista*, *Bovistella*, *Calvatia*, *Disciseda*, *Geaster* and *Lycoperdon*. No clamp connections have been observed, either in the vegetative mycelium or in the mycelium of the tissues of the fructification.

All photographs by H. Drake; drawings and photomicrograph by the writer.